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Alternaria cerasidanica sp. nov., isolated in Denmark from drupes of *Prunus avium*

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Abstract — The ex-type strain of *Alternaria cerasidanica* was isolated in 2001 from an immature, asymptomatic drupe of *Prunus avium* collected at a commercial cherry orchard near Skælskør, Denmark. Cultural morphology, sporulation pattern and cluster analyses of combined RAPD, RAMS (microsatellite), and AFLP fingerprints of *A. cerasidanica* and 167 strains of *Alternaria* spp. support the placement of *A. cerasidanica* within the *A. infectoria* species-group sensu Simmons and its segregation from other members of this group. *A. cerasidanica* is currently monotypic and known only from preharvest sweet cherry fruit in Denmark.

Key words — hyphomycetes

Introduction

During studies of fungal colonization of drupes of *Prunus avium* (L.) L. (sweet cherry) Dugan & Roberts (1994) consistently isolated *Alternaria* strains from gynoecial scar tissues on immature cherry fruit collected at several locations in Washington State. Many of these isolates were subsequently characterized according to their sporulation pattern observable at 50× and by cluster analysis of RAPD amplicons (Roberts et al. 2000). Notable amongst the hundred of *Alternaria* isolates so obtained was the complete absence of isolates exhibiting cultural and morphological characters of *A. infectoria* species-group. A subsequent opportunity to examine cherry fruit from a geographically isolated (from Washington state) cherry orchard in Denmark was taken to determine if the absence of *A. infectoria* species-group isolates was also characteristic of Danish populations of *Alternaria* associated with developing cherry fruit.

Materials and methods

Conditions of isolation, culture and observation

During June–July 2001, RGR and BA harvested immature sweet cherry fruit (cv. Van) from a commercial orchard near Skælskør, Denmark. Harvested cherry fruit were processed by methods previously described (Roberts & Dugan 1994); they were transported on ice to DTU (Lyngby), surface disinfested with sodium hypochlorite, then the style and sepal scar tissues were aseptically excised and plated onto PCA. One hundred and four representative isolates were preserved as lyophilized conidial suspensions held at –20 C or as colonized agar blocks held in sterilized distilled water at 4 C. The media, methods and conditions for growth and observation of the resulting *Alternaria* isolates followed Simmons & Roberts (1993). Approximately 100 isolates of *Alternaria* were obtained and characterized by the pattern of sporulation evident at 50× magnification with a dissecting microscope. RGR 01.0149 was among the isolates that exhibited the diagnostic sporulation patterns and cultural characters of the *A. infectoria* species-group. To observe and record microscopic characters double-stick tape was pressed onto colony surfaces and then mounted spore-side-up in several drops of lactic acid on a microscope slide for observation at higher magnifications. Microscopic morphology was observed at 200–630× and recorded digitally using a Zeiss Axiocam MRc5 camera and a Zeiss Axioplan microscope. Color references in the taxonomic descriptions follow the Methuen Handbook of Colour (Kornerup & Wanscher 1989).

Molecular analysis

Selected cultures were grown, DNA was isolated and prepared, and combined fingerprints from randomly amplified polymorphic DNA (RAPD), randomly amplified microsatellites (RAMS) and amplified fragment length polymorphism (AFLP) analyses were generated and analyzed by methods reported previously (Roberts et al. 2000, Roberts 2007). The band-based fingerprint data from the Danish *A. infectoria*-group isolates were added to an existing database of molecular characters in BioNumerics 5.10 (Applied Maths, Ghent, Belgium), then analyzed by cluster analysis using the 'Similarity' and 'Average From Experiments' settings.

Taxonomic description

Alternaria cerasidanica R.G. Roberts, **sp. nov.** Fig. 1

MycoBank MB 513487

Ex cultura in agaro PCA post 7 dies, temperatura 21 C descripta. Coloniae ca. 59 mm diam., griseobrunneae, indistincte concentrice zonatae. Conidiophora primaria simplicia vel ramosa, geniculata, 40–167 × 3.7–7.0 µm. Conidia 4–5 (vel num. minor quam 10) catenata, subglobosa vel ellipsoidea vel subcylindrica, erostrata (vel obclavata, pseudorostrata in catenis), 23–70 × 11–25 µm, transverse 2–8 euseptata, 0–4 longiseptata, conidiophoris secondariis apicalibus et lateralibus 5.8–86.7 × 3.6–7.0 µm. Conidia laevia vel plerumque punctulata vel verrucosa vel tuberculata, olivaceobrunnea, septis atrobrunneis. Teleomorphosis ignota.

Type (**holotype**): BPI 878241; (dried PCA culture preparation ex RGR 01.0149), isol. RG Roberts from an immature drupe of *Prunus avium*, June 2001, Skælskør, Denmark.

Figure 1. Conidia and conidiophores of *A. cerasidanica* from a 7-day-old PCA culture. Bar = 50 µm.

Ex-type culture deposited at Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands as CBS 121923.

Etymology: *cerasus* + *danica* – cherry fruit in Denmark as source of isolate

Description from cultures grown on PCA for 2–7 days at 25 C, 21% RH, and an 8-hour photoperiod. Colonies at 4 days ca 45 mm diam., azonate, with an even, granular appearance, centrally golden brown near 5B-DF6 with hyaline, asporogenous margins ca 8mm wide. Colonies at 7 days ca 59 mm diam., grayish brown near 6F3, indistinctly zonate, with a low, even turf of pigmented (olive, near 3D4-3E4 by transmitted light), funiculose sporogenous hyphal elements from which simple or branched primary conidiophores develop. Colony

zonations produced during light exposure result from primary conidiophores produced from the agar surface as well as aerial hyphae. Conidia are present without colony scarification, but sporulation density is greatly increased after scarification. Primary conidiophores 40.0–167.0 × 3.7–7.0 μm, pale to light grey (1B1-1D1) by transmitted light, branched or unbranched, often becoming geniculate from successive production of conidia, developing as lateral outgrowths from pigmented, funiculose aerial hyphae or from hyphae embedded within the agar medium. At 50×, conidia appear ellipsoidal to lenticular, robust, dark (opaque), in sparingly branched chains. Conidia developing in chains of less than 10 conidia, commonly four to five conidia per chain. Conidia 23.0–70.0 × 11.0–25.0 μm, erostrate, becoming pseudorostrate with chain formation, subglobose to ellipsoidal when young, occasionally becoming obclavate as the upper spore body elongates into a secondary conidiophore. Spore bodies may appear smooth in median optical section, but are usually ornamented, from punctulate to verrucose to coarsely tuberculate, especially on the proximal third of the spore body. Spore bodies near olive brown (4E4) by transmitted light. Secondary conidial chains produced by sympodial development of apical or lateral, short to elongate, geniculate, usually unbranched secondary conidiophores 5.8–86.7 × 3.6–7.0 μm, pigmented as primary conidiophores, frequently with an enlarged apex. Basal conidia are frequently long ellipsoidal to subcylindrical, in maturity with 2–8 darkly pigmented transsepta and 0–4 longisepta, which may be as dark as the transverse eusepta or less conspicuously pigmented. Neither ascomata nor ascospores were observed in culture or on agar blocks held in refrigerated water. After two days of growth, the appearance of the field at 50× is dominated by the large, solitary broadly ellipsoidal to ovoid conidia. By day 4, short chains of 3–4 conidia can be seen but robust, solitary ellipsoidal conidia still predominate. *A. cerasidanica* is known only from the ex-type culture.

Discussion

Although *A. cerasidanica* is newly described here, the key to species within the *A. infectoria* species-group of Simmons (2007) is still useful to narrow the possible choices and thus differentiate it from the other species accepted by Simmons. As Simmons' key to the group does not resolve *A. photistica* and *A. cerasidanica*, the ex-type cultures of *A. photistica* (EGS 35.172) and *A. cerasidanica* (RGR 01.0149) were compared. Several of the agar blocks stored in water from which *A. photistica* was recovered bore senescent pseudothecia and viable ascospores of *Lewia photistica* E.G. Simmons 1986, but no intact asci were observed, confirming Simmons' observation and note on the culture (Simmons 2007). As noted previously, similarly stored agar blocks from which *A. cerasidanica* was recovered did not bear evidence of a teleomorphic state.

Figure 2. First-formed conidia from 2-day-old PCA cultures of *A. photistica* (EGS 35.172, above) and *A. cerasidanica* (RGR 01.0149, below). Bar = 50 µm.

The largest conidia of *A. photistica* observed under our growth conditions were seen after two days growth on the agar block after transfer from the water storage tube to PCA. The largest of these first-borne conidia were 53–108 × 13–21 µm (Fig. 2) and were considerably larger than 50 µm given in the type description (Simmons 1986b). Conidia produced thereafter were smaller, 31–56(–72) × 11–18 µm (Fig. 3).

The reader is initially directed to Section K (in Simmons 2007: 586) as the conidia of *A. cerasidanica* occur in branching chains and develop conspicuous

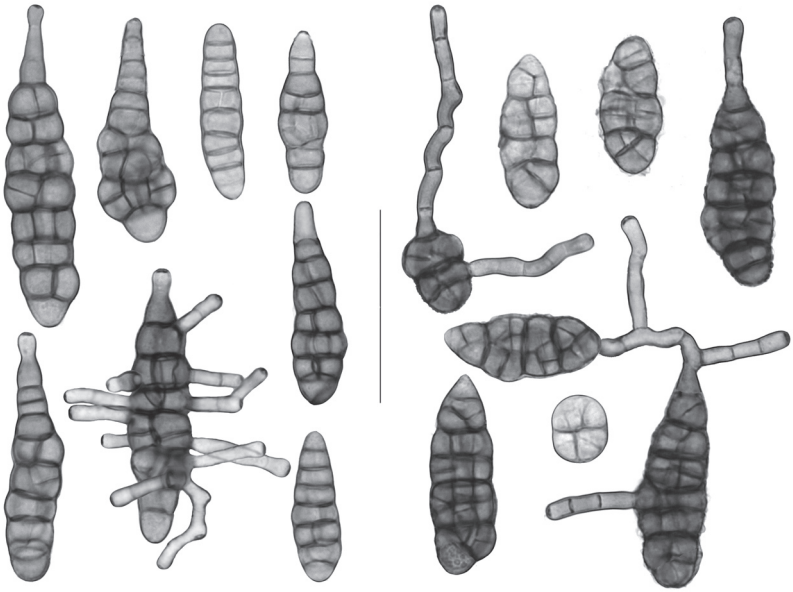

Figure 3. Conidia from 14-day-old PCA cultures of *A. photistica* (left of bar) and *A. cerasidanica* (right of bar). Bar = 50 µm.

secondary conidiophores that determine in part the spatial characteristics of the sporulation apparatus. Young cultures, although sporogenous, tend to be dominated by aerial funiculose hyphae that are sparingly conidial compared to the densely packed lawn of conidiophores and conidia that develop within 24 h after colony scarification. The characters of *A. cerasidanica* that lead one through the choices in this Section are 1) conidia are ovoid to ellipsoid (to choice 4), 2) conidium color is stable in liquid mounts (to choice 5), 3) seta-like or arborescent elements are lacking (to choice 7), conidium length to max. 70–160 µm (to choice 8), and conidium width max. to range 15–20(–25) µm (to choice 9). At choice 9, *A. cerasidanica* is excluded from both *A. avenicola* and *A. triticicola* by virtue of the smaller size of the largest conidia.

Conidial size ranges overlap for *A. photistica* and *A. cerasidanica*, but in freshly prepared lactic acid mounts these species differ in conidium color, septation, shape, and ornamentation. By transmitted light *A. photistica* conidia and conidial septa are relatively pale and straw-colored (straw yellow (3B4) to grayish orange (5B3)) whereas conidia of A. *cerasidanica* are darker, approaching olive brown (4E4) and more coarsely ornamented, becoming coarsely punctate

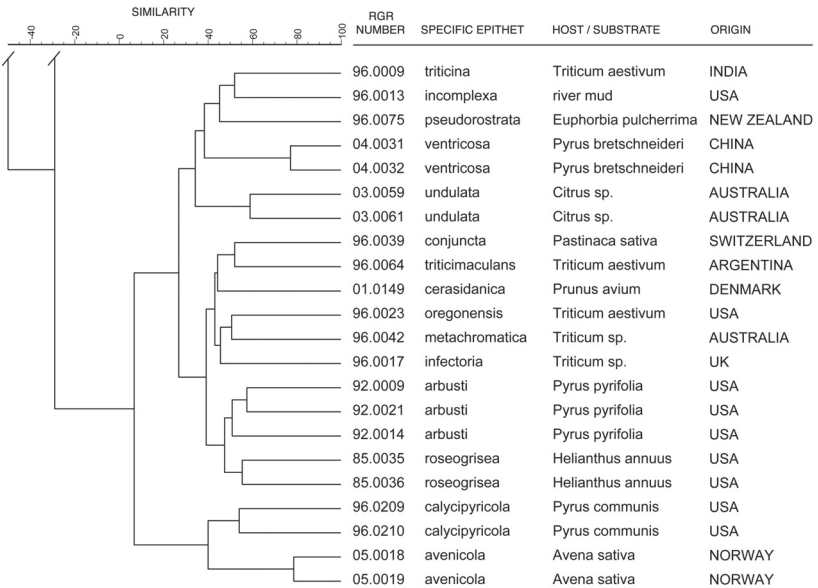

Figure 4. The *A. infectoria* species-group cluster excerpted from a cluster analysis of a combined RAPD, RAMS and AFLP fingerprint data set for each of 167 isolates of *Alternaria* spp. from various hosts, substrates, geographic origin, and sporulation pattern groups.

in age. Conidial septa of *A. photistica* are relatively undifferentiated, remain unthickened and lightly pigmented well into development, and regularly constrict the outline of the conidia. Conidial cross-septa in A. *cerasidanica* are precociously and darkly pigmented at two days growth, appearing thickened and nearly opaque in maturity (Fig. 2), almost embellisioid. The distal spore bodies of *A. photistica* often gradually taper to a blunt, rounded apex of 2–4 cells that is usually abruptly narrower than the proximal spore body. The distal ends of conidia from *A. cerasidanica* taper abruptly to a pyramid-shaped apex, which usually involves only one or two cells. With age, the largest conidia of *A. photistica* may bristle with short secondary conidiophores, but similar development is not observed with *A. cerasidanica* (Fig. 3). Conidial walls of *A. photistica* appear translucent with no or finely granulate ornamentation, whereas those of *A. cerasidanica* become coarsely punctate and darkly pigmented in age (Fig. 3).

An excerpt from a band-based cluster analysis of combined RAPD, RAMS and AFLP fingerprints presented in Fig. 4 provides supporting molecular evidence for the morphologically-based assignment of *A. cerasidanica* to the *A. infectoria* species-group and supports its segregation from the other included

species in our *Alternaria* database. All *Alternaria* isolates in the present study previously assigned to the *infectoria* species-group and the anamorphic states of *Lewia* clustered together in this branch.

Isolation of several *infectoria* species-group strains from Danish cherry fruits in this study is notable, as Dugan & Roberts (1994) reported no *infectoria* species-group isolates from among many hundreds of *Alternaria* strains isolated from thousands of pre-harvest cherry fruit in Washington State. Eight of the 104 isolates obtained from Danish cherry fruit belong in the *infectoria* species-group based upon cultural morphology and sporulation pattern observed at 50×, a statistic made relevant by their aforementioned absence from cherry fruit sampled in Washington State. The seven remaining *infectoria* species-group isolates from Danish cherry include several other undescribed taxa not treated here.

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